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Radio frequency electric fields processing of orange juice☆

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Abstract

The non-thermal process of radio frequency electric fields (RFEF) has been shown to inactivate bacteria in apple juice at moderately low temperatures, but has yet to be extended to inactivate bacteria in orange juice. An 80 kW RFEF pasteurizer was used to process pulp-free orange juice at flow rates of 1.0 and 1.4 l/min. *Escherichia coli* K12 in orange juice was exposed to electric field strengths of 15 and 20 kV/cm at frequencies of 21, 30, and 40 kHz. Ascorbic acid (Vitamin C) content and color of the juice before and after treatment were analyzed. Electrical energy costs were calculated using the measured voltage and current. An energy balance was performed using the inlet and outlet temperatures. Processing at an outlet temperature of 65 °C reduced the population of *E. coli* by 3.3 log relative to the control. Increasing the treatment time and temperature and decreasing the frequency enhanced the level of inactivation. Varying the electric field strength over the range of conditions used had no effect on the inactivation. No loss in ascorbic acid or enzymatic browning was observed due to RFEF processing. The electrical energy determined using the voltage and current was 180 J/ml. This was in good agreement with the energy calculated using the temperature data. The electrical cost was $0.0026/l of orange juice. The results provided the first evidence that the RFEF process inactivates bacteria in orange juice at moderately low temperatures.

Keywords: Radio frequency electric fields; Orange juice; Non-thermal pasteurization; Quality; *E. coli*; Processing costs

Industrial relevance: The RFEF process has been shown to inactivate *E. coli* in apple juice at moderately low temperatures, but has yet to be extended to inactivate bacteria in orange juice. An RFEF pilot plant pasteurizer was used to process orange juice at rates of up to 1.4 l/min. RFEF processing reduced the population of *E. coli* by 99.3% at 60 °C and a hold time of 3 s, whereas conventional heating at the same conditions had no effect on the *E. coli*. This work demonstrated that the non-thermal RFEF process can be extended to inactivate bacteria in orange juice.

1. Introduction

Seventy three percent of respondents in a recent survey regarded nutrition as the most important factor in determining which juice to buy (Roberts, 2003). While thermal pasteurization of orange juice significantly reduces the risk of foodborne disease, it can reduce the nutrient content as well. Therefore, many consumers choose to drink unpasteurized orange juice. In July 2005, the Food and Drug Administration issued a nationwide warning to consumers against drinking unpasteurized orange juice products because they have the potential to be contaminated with *Salmonella typhimurium* and have been associated with an outbreak of human disease caused by this organism (FDA, 2005). Fifteen cases of illness were directly linked to a history of consumption of unpasteurized orange juice from mid-May to June 2005. Prior to this, a *Salmonella enteritidis* outbreak in 2000 was caused by unpasteurized orange juice and resulted in 88 illnesses in six Western states. A *Salmonella muenchen* outbreak in 1999 was caused by unpasteurized orange juice and resulted in 423 illnesses in 20 states and 3 Canadian provinces and contributed to one death (FDA, 2001).

The radio frequency electric field (RFEF) non-thermal process has recently been developed for inactivating bacteria in apple juice (Geveke & Brunkhorst, 2004a,b). In an electric field, a voltage is formed across the cell membrane (Zimmermann, 1986). The opposite charges on either side of the membrane are attracted to each other and the membrane

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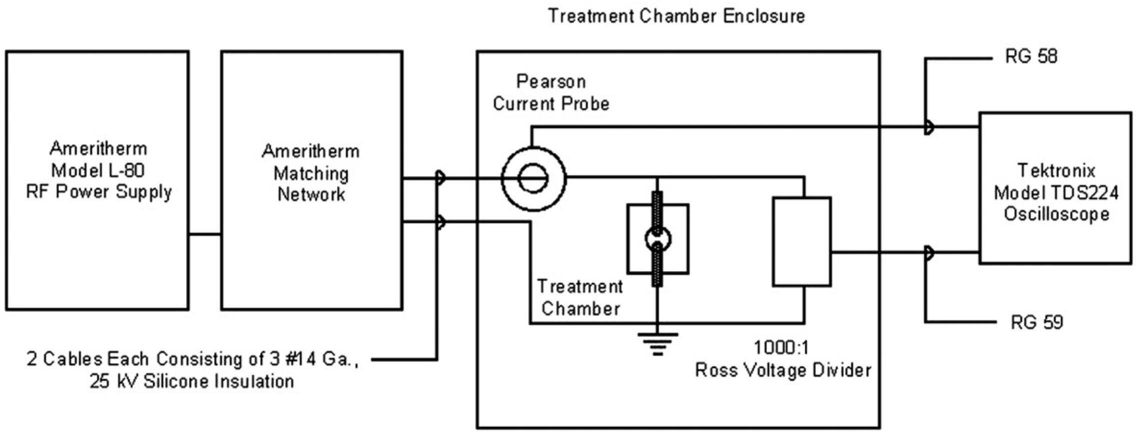

Fig. 1. Electrical diagram of radio frequency electric fields system.

becomes thinner. At field strengths greater than approximately 5 kV/cm, pores are formed in the membrane and the cell ruptures. Treatment times are less than 1 ms. The electric field also raises the temperature of the juice by ohmic heating due to the electrical resistance of the juice. The final temperature is less than 70 °C and the juice is cooled within several seconds using a heat exchanger. The combination of lower time and temperature enables the juice to retain maximum nutrients. The RFEF process is similar to the pulsed electric field (PEF) process. The difference is that in PEF processing, the high voltage is applied in pulses using a pulse generator, whereas in RFEF processing, the voltage is applied continuously using an AC generator.

RFEF processing at 30 kV/cm and 20 kHz reduced the population of *Saccharomyces cerevisiae* in water by 3.8 log (99.98%) at 35 °C (Geveke & Brunkhorst, 2003). RFEF processing at 21 kV/cm and 55 °C inactivated *Escherichia coli* K12 in apple juice by 1.9 log (98.7%) relative to the control (Geveke & Brunkhorst, 2004a). Raising the temperature increased inactivation. Radio frequencies of 15 and 20 kHz inactivated *E. coli* better than frequencies of 30–70 kHz. The flow rate was limited to 0.55 l/min by the RF power supply. The RFEF process was successfully scaled up from 0.55 l/min to 1.4 l/min using an innovative pilot plant consisting of an 80 kW power supply and novel matching network (Geveke & Brunkhorst, 2004b). RFEF processing reduced *E. coli* in apple juice by 2.7 log at 60 °C and a hold time of 3 s, whereas conventional heating at the same conditions had no effect. Non-thermal inactivation of *E. coli* K12 was dependent upon the electric field strength, frequency, treatment time and temperature.

The objective of this work was to extend the RFEF process to orange juice. Orange juice has a substantially higher conductivity than apple juice; hence, it requires greater processing power. An additional objective was to analyze the color and ascorbic acid content before and after RFEF processing which had not been done before.

2. Materials and methods

The RFEF processing research was performed in a food pilot plant, so pathogenic bacteria could not be used. *E. coli* K12 substrain C600 (Fratamico, Bhaduri, & Buchanan, 1993) was maintained on tryptose agar (Difco Laboratories, Franklin Lakes, NJ) at 4 °C. The *E. coli* was cultured in brain heart infusion (Difco Laboratories) for 24 h at 37 °C. Florida's Natural Premium (not from concentrate) pulp-free orange juice was purchased from a local supermarket. The juice was inoculated from the stationary phase culture to give an initial level of *E. coli* of approximately 6 log cfu/ml. The pH and conductivity of the juice were 3.9 and 4.14 mS/cm, respectively.

Geveke and Brunkhorst (2004b) have previously described the radio frequency electric fields (RFEF) power supply system used in this investigation. It consisted of an 80 kW RF power supply (Ameritherm, Scottsville, NY, model L-80) and a custom designed matching network that enabled the RF energy to be applied to a resistive load (Ameritherm) over a frequency range of 21.1 to 40.1 kHz (Fig. 1). The RFEF power supply was connected to a pair of treatment chambers connected in series. The chambers were made of Rexolite, a transparent cross-linked polystyrene copolymer (C-Lec Plastics, Philadelphia, PA). The treatment chambers were designed to converge the orange juice into a narrow flow area in order to reduce the power requirement (Geveke & Brunkhorst, 2004a; Matsumoto, Satake, Shioji, &

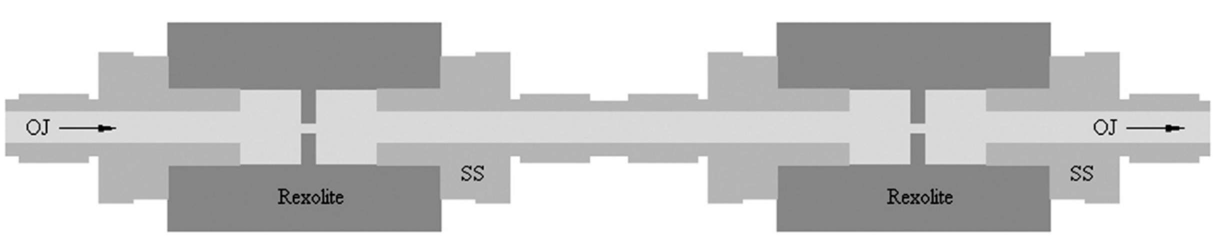

Fig. 2. Cross-section of two radio frequency electric fields converged treatment chambers in series including Rexolite insulation and stainless steel (SS) electrodes.

Sakuma, 1991; Sensoy, Zhang, & Sastry, 1995). Juice entered and exited the Rexolite chambers through the annuli of cylindrical stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR) as shown in Fig. 2. Between the electrodes in each treatment chamber, there was a thin partition with a channel of circular cross-section through the center. The diameter and length of the channel were 0.12 cm and 0.20 cm, respectively. A 0.9 cm space between the end of each of the electrodes and the central channel prevented arcing. The two treatment chambers were connected by a 2.8 cm length of stainless steel tubing. The output of the RFEF power supply was connected to the tubing between the treatment chambers and the outer electrodes were grounded. The maximum voltage applied was limited to 4.0 kV_{peak} in order to control the temperature rise of the orange juice. Therefore, the nominal maximum electric field strength used in the study was 20 kV/cm obtained by dividing the peak voltage, 4.0 kV, by the length of the central gap, 0.2 cm. The minimum voltage applied was 3.0 kV_{peak} and was limited by the RF power supply and matching network operating requirements. Hence, the minimum electric field strength used in the study was 15 kV/cm.

The supplied voltage and current to the RFEF treatment chambers were measured using an oscilloscope (Tektronix, Beaverton, OR; model TDS224), current probe (Pearson Electronics, Palo Alto, CA, model 411), and a voltage divider (Ross Engineering, Campbell, CA; model VD15-8.3-A-KB-A).

QuickField™ (Tera Analysis Ltd, Svendborg, Denmark, version 5.0) finite element analysis software was used to model the anisotropic electric field strength within the treatment chamber. The results of the model indicated that the orange juice flows through the electrode and enters a field-free region. It then flows into the cylindrical gap where the field is quickly raised to the maximum field strength. The field within the gap is nearly uniform which ensures that all of the juice is treated equally. The uniformity improves the energy efficiency of the process. By minimizing the regions within the treatment chamber where the electric field is too low to inactivate bacteria and only heats the juice, approximately less than 5 kV/cm, the energy loss is minimized. Similarly, by minimizing the regions where the field is higher than needed to inactivate bacteria, the energy loss is minimized. Thus, the outlet temperature is lessened and the orange juice is not over heated.

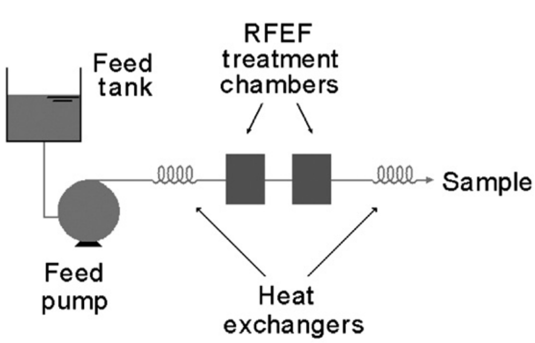

Fig. 3. Schematic diagram of continuous radio frequency electric fields process including two treatment chambers in series.

The experimental system included a stainless steel feed tank and a progressing cavity pump (Moyno, Springfield, OH; model 2FG3) that supplied orange juice to the RFEF system through stainless steel tubing as shown in Fig. 3. The minimum flow rate was 1.0 l/min in order to control the temperature rise of the orange juice. The maximum flow rate was restricted to 1.4 l/min because of the system's pressure drop and pump limitations. Turbulent flow within the treatment chambers (Reynolds Number > 18,000) improved the processing uniformity. A back pressure of 1 atmosphere gauge minimized arcing. The juice was exposed to intense RFEF in the chambers for a total duration of 270 µs at 1.0 l/min and 190 µs at 1.4 l/min. At a frequency of 21.1 kHz and a flow rate of 1.4 l/min, the juice was exposed to 2 AC cycles of RFEF in each chamber. The inlet temperature to the RFEF treatment chamber was controlled using a 0.24 m^2 stainless steel heat exchanger (Madden Manufacturing, Elkhart, IN; model SC0004) and a temperature controller (Cole-Parmer, model CALL 9400).

The temperatures of the orange juice before and after the RFEF treatment chambers were measured with 3.2 mm diameter chrome–constantan thermocouples (Omega Engineering, Inc., Stamford, CT). The temperatures were continuously logged to a data acquisition system (Dasytec USA, Amherst, NH, Dasylab version 5.0). The outlet temperatures ranged from 50 to 65 °C.

The orange juice was quickly cooled after exiting the treatment chambers to less than 25 °C using a stainless steel heat exchanger sample cooler (Madden Manufacturing, model SC0004). The lengths of time for the juice to travel from the treatment chamber to the sample cooler were 3 and 2 s, respectively for the 1.0 and 1.4 l/min flow rates.

Controls were performed to determine the antimicrobial effect of temperature alone. In order to ensure that the control juice received the same time and temperature history as the treated juice, the high fields, converged treatment chambers were replaced with an ohmic heating chamber. The chamber consisted of two stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR) inserted into a 10.2 cm length of 0.64 cm ID plastic tubing. The ohmic heater quickly brought the juice temperature up to the desired temperature. The control juice was identically held for either 2 or 3 s before cooling to less than 25 °C.

The feed and product samples were appropriately diluted with sterile 1% peptone water and were plated on tryptose agar using a spiral plater (Spiral Biotech, Bethesda, MD; model Autoplate 4000) and incubated at 37 °C for 24 h. Enumerations were made with a colony counter (Spiral Biotech, model CASBA 4).

Samples of orange juice were taken before and after RFEF processing and were analyzed for browning and ascorbic acid. Vitamin C (ascorbic acid) was measured using an HPLC method as described earlier (Fan, Thayer, & Handel, 2002). Orange juice was centrifuged at 12,000 *g* for 10 min at 5 °C in a Sorvall RC2-B refrigerated centrifuge (Kendro Laboratory Products, Newtown, CT). The supernatant was filtered through a 0.45 µm Acrodisc LC 13 PVDF syringe filter (Gelman Sciences, Ann Arbor, MI) before being analyzed using a

Hewlett-Packard Ti-series 1050 HPLC system (Agilent Technologies, Palo Alto, CA.). The HPLC system consisted of an autosampler, an integral photodiode-array detector, an auto-injector and a Hewlett-Packard Rev. A02.05 Chemstation. Injection volume was 20 µl. Separation of compounds was achieved with an Aminex HPX-87H organic acids column (300 × 7.8 mm) fitted with a microguard cation H+ eluted with a mobile phase of 5 mM sulfuric acid at a flow rate of 0.5 ml/min. Column temperature was maintained at 30 °C using a column heater (Bio-Rad Laboratories, Hercules, CA). Ascorbic acid was monitored at 245 nm and calculated from an ascorbic acid standard. To measure browning, orange juice was centrifuged at 12,000 *g* for 10 min at 5 °C (Fan & Thayer, 2002). The absorbance of the supernatant at 420 nm was measured using a spectrophotometer (Shimadzu UV-1601 spectrophotometer, Shimadzu Scientific Instruments, Columbia, MD).

Each RFEF experiment was performed in duplicate. Results were expressed as the means of these values ± the standard deviations calculated using Microsoft Excel statistical analysis algorithms.

3. Results and discussion

RFEF processing successfully inactivated *E. coli* K12 in orange juice at non-thermal conditions. The extent of microbial inactivation is dependent on the treatment time, frequency and temperature and is independent of electric field strength over the narrow range of conditions studied.

A series of experiments were performed at 21.1 kHz to determine the effects of electric field strength and temperature on inactivation. The population of *E. coli* in orange juice was reduced by 2.1 ± 0.4 log after being exposed to a 15 kV/cm peak electric field at a treatment time of 270 µs, outlet temperature of 60 °C, and hold time of 3 s (Fig. 4). When the juice was ohmicly heated at the same frequency, 21.1 kHz, to the same outlet temperature, 60 °C, and held for the same time, 3 s, the population of *E. coli* was unaffected. The non-thermal inactivation is believed to be due to electroporation of the cells as a result of high electric fields (Zimmermann, Pilwat, & Rieman, 1974). These results compare favorably to those obtained using the same RFEF equipment to process apple juice (Geveke & Brunkhorst, 2004b). The population of *E. coli* in apple juice was reduced by 1.3 log after being exposed to a 15 kV/cm peak electric field at a treatment time of 270 µs, outlet temperature of 60 °C, and hold time of 3 s. The results of the present study successfully extend the application of non-thermal RFEF processing to orange juice.

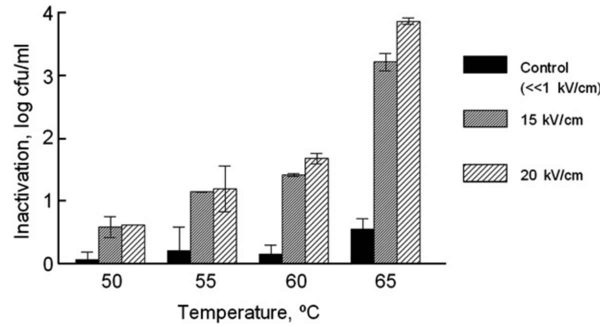

Fig. 5. Effects of outlet temperature and radio frequency electric field strength on the inactivation of *E. coli* at 190 µs treatment time and 2 s hold time (1.4 l/min flow rate). Means of two replicate experiments.

Inactivation increased as the temperature increased from 50 to 65 °C. The inactivation rose from 1.6 ± 0.5 log to 3.6 ± 0.3 log at a treatment of 15 kV/cm. Although there was some thermal inactivation at 65 °C, the vast majority of the RFEF inactivation at 65 °C was due to non-thermal effects. There was no effect on inactivation of varying the field strength within the range of 15 to 20 kV/cm. Typically in RFEF and PEF processing, inactivation is dependent on field strength (Barsotti & Cheftel, 1999; Geveke & Brunkhorst, 2003, 2004b). However, a similar result to that observed in this study was reported for RFEF processing of apple juice (Geveke & Brunkhorst, 2004a). Inactivation increased as the electric field strength increased up to 16 kV/cm. However, inactivation remained constant with field strength above 16 kV/cm, especially at 45 °C and 50 °C. Jayaram, Castle, and Margaritis (1992) applied PEF to *Lactobacillus brevis* and observed similar behavior. Inactivation of *L. brevis* greatly increased with field strength up to 15 kV/cm, whereas, at higher fields, inactivation remained constant at temperatures between 30 and 45 °C. Wouters, Dutreux, Smelt, and Lelieveld (1999) reported similar results for PEF treatment of *Listeria innocua* except that the threshold field strength was higher, 30 kV/cm, and was observed between 45 °C and 60 °C.

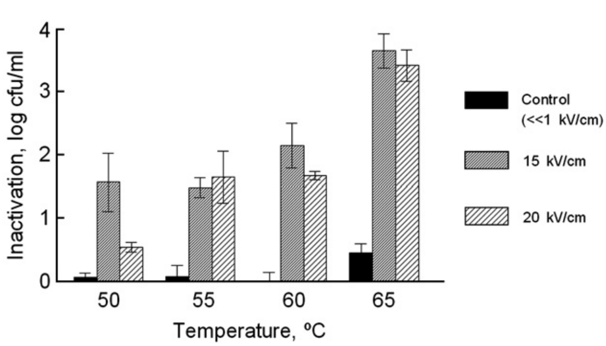

Fig. 4. Effects of outlet temperature and radio frequency electric field strength on the inactivation of *E. coli* at 270 µs treatment time and 3 s hold time (1.0 l/min flow rate). Means of two replicate experiments.

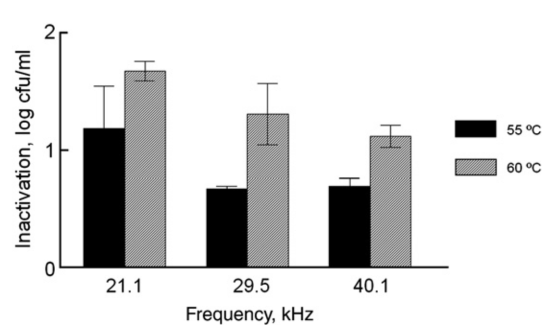

Fig. 6. Effects of electric field frequency and outlet temperature on the inactivation of *E. coli* at 20 kV/cm, 270 µs treatment time and 3 s hold time. Means of two replicate experiments.

Increasing the orange juice flow rate to 1.4 l/min, and correspondingly decreasing the treatment time to 190 μs and the hold time to 2 s, lessened the treatment effectiveness (Fig. 5). The electrical energy costs were calculated for the case of RFEF processing of orange juice at 1.4 l/min, 20 kV/cm, and an outlet temperature of 65 °C. At these conditions, the population of *E. coli* was reduced by 3.9±0.1 log. Based on the voltage and current measured by the oscilloscope, 4.0 kV_{peak} and 2.1 A_{peak}, respectively, the energy applied was 180 J/ml. From the inlet temperature, 26 °C, the energy calculated to raise the temperature of the orange juice to 65 °C is 160 J/ml. This is in fairly good agreement with the energy calculated using current and voltage. The estimated energy required for a 5 log reduction using PEF ranges from 100 to 400 J/ml (Barsotti & Cheftel, 1999; Schoenbach, Katsuki, Stark, Buescher, & Beebe, 2002). It is probable that the RFEF electrical costs for a 5 log reduction will be similar to those of PEF as they are both considered electroporation processes (Geveke & Brunkhorst, 2004a). Based on the U.S. Department of Energy's data for the average industrial electric price for the first four months of 2004 of $0.0514/kWh, the energy cost for the RFEF process was $0.0026/l of orange juice. For comparison, the energy costs for conventional thermal pasteurization, with heat regeneration or recovery, are $0.00063/l or less.

The inactivation of *E. coli* in orange juice increased as the frequency was decreased from 40.1 kHz to 21.1 kHz as shown in Fig. 6. These results agree with those obtained using the same RFEF equipment to process apple juice (Geveke & Brunkhorst, 2004b). In another work, greater inactivation of *E. coli* in apple juice occurred at frequencies of 15 and 20 kHz compared to frequencies of 30 to 70 kHz (Geveke & Brunkhorst, 2004a). These results are interesting, not only because they indicate that the RFEF process could be more efficient at even lower frequencies, but also because the RFEF equipment costs would be significantly less if the industrially common frequency of 60 Hz could be used. A disadvantage of 60 Hz is that it is more dangerous to work with than higher frequencies because electricity can penetrate the human body deeper at lower frequencies. However, sufficient safety features can be designed into a 60 Hz system to prevent injury.

Two of the commonly occurring degradations in juice quality are non-enzymatic browning and loss of ascorbic acid. An experiment was conducted to ascertain the effect of RFEF processing on these two aspects of juice quality. Orange juice was processed at 20 kV/cm and an outlet temperature of 65 °C with a hold time of 2 s. At these conditions, the population of *E. coli* was reduced by 3.9±0.1 log. Many fruit juices are rich in ascorbic acid (Vitamin C). Ascorbic acid is, however, sensitive to many processing and storage conditions. It is known that exposure to high temperatures during pasteurization results in a considerable loss of ascorbic acid. For example, pasteurization (90 °C for 60 s) of fresh orange juice resulted in a 2.4% loss in ascorbic acid (Roig, Rivera, & Kennedy, 1995). No loss in ascorbic acid was observed due to RFEF processing (Table 1), probably due to the low treatment time and temperature. Uemura and Isobe (2003) used a 20 kHz RFEF apparatus to study inactivation of *Bacillus subtilis* spores in orange juice. The orange juice was RFEF processed at 121 °C under pressurized conditions to elevate the boiling point. A 16.3 kV/cm field reduced the viable *B. subtilis* spores by 4 log in <1 s of treatment. Only 10% of the original ascorbic acid in the orange juice was destroyed after RFEF treatment. In the present study, the juice was RFEF processed at only 65 °C.

Table 1
Effect of radio frequency electric fields (RFEF) processing on the level of ascorbic acid and color

Sample	Ascorbic acid (μg/ml)	Color
Unprocessed	235±15	0.097±0.014
RFEF processed	240±10	0.104±0.013

Processing conditions were 20 kV/cm and an outlet temperature of 65 °C with a hold time of 2 s (n=2).

Non-enzymatic browning is due to Maillard-type reactions of sugars, amino acids and ascorbic acid. The reactions, influenced by many factors (such as temperature and oxygen), not only lead to browning and loss of ascorbic acid, but also produce compounds that contribute to off-flavor of juice. The oxidation of ascorbic acid can play an important role in the browning of fruit juice. No change in brownness of orange juice was observed as a result of RFEF processing (Table 1), coinciding with the complete retention of ascorbic acid.

Additional studies are recommended. The RFEF process needs to be further scaled up to be of commercial interest. This should be possible by designing larger treatment chambers and installing larger pumps and heat exchangers. The greater flow rates will necessitate greater processing power, but the RFEF 80 kW power supply should be able to handle this. An additional goal is to achieve a 5 log reduction. This should be attainable by adding several more treatment chambers in series. Also, RFEF processing and thermal processing of fresh orange juice should be performed in order to evaluate the quality improvement of RFEF processing due to shorter time and lower temperature. Finally, RFEF processing at lower frequencies, where the efficiency may be enhanced, deserves attention.

4. Conclusions

The radio frequency electric fields (RFEF) process was capable of inactivating *E. coli* K12 in orange juice at non-thermal conditions. RFEF processing at an outlet temperature of 60 °C reduced the population of *E. coli* by 2.1 log, whereas thermal processing at the same conditions had no effect. The level of inactivation was dependent on the outlet processing temperature and treatment time and was inversely dependent on the frequency. There was no difference in the level of inactivation at electric field strengths of 15 and 20 kV/cm. The electrical energy cost for the RFEF process, that reduced the population of *E. coli* by 3.9±0.1 log, was $0.0026/l of orange juice. Neither loss in ascorbic acid (Vitamin C) nor enzymatic browning was observed due to RFEF processing. The RFEF process should be capable of pasteurizing orange juice at moderately low temperatures by increasing the number of treatment stages.

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References

Barsotti, L., & Cheftel, J. C. (1999). Food processing by pulsed electric fields. II. Biological aspects. *Food Reviews International*, *15*(2), 181−213.

Fan, X., & Thayer, D. W. (2002). Gamma radiation influences browning, antioxidant activity and malondialdehyde level of apple juice. *Journal of Agricultural and Food Chemistry*, *50*, 710−715.

Fan, X., Thayer, D. W., & Handel, A. P. (2002). Nutritional quality of irradiated orange juice. *Journal of Food Processing and Preservation*, *26*(3), 195−211.

FDA. (2001). FDA publishes final rule to increase safety of fruit and vegetable juices. *Press Release P01-03.*

FDA. (2005). FDA issues nationwide health alert on Orchid Island unpasteurized orange juice. *Products Recalls, Market Withdrawals and Safety Alerts.*

Fratamico, P. M., Bhaduri, S., & Buchanan, R. L. (1993). Studies on *Escherichia coli* serotype O157:H7 strains containing a 60-MDa plasmid and on 60-MDa plasmid-cured derivatives. *Journal of Medical Microbiology*, *39*(5), 371−381.

Geveke, D. J., & Brunkhorst, C. (2003). Inactivation of *Saccharomyces cerevisiae* using radio frequency electric fields. *Journal of Food Protection*, *66*(9), 1712−1715.

Geveke, D. J., & Brunkhorst, C. (2004a). Inactivation of *Escherichia coli* in apple juice by radio frequency electric fields. *Journal of Food Science*, *69* (3), 134−138.

Geveke, D. J., & Brunkhorst, C. (2004b). RFEF pilot plant for inactivation of *Escherichia coli* in apple juice. *Fruit Processing*, *14*(3), 166−170.

Jayaram, S., Castle, G. S. P., & Margaritis, A. (1992). Kinetics of sterilization of *Lactobacillus brevis* cells by the application of high voltage pulses. *Biotechnology and Bioengineering*, *40*(11), 1412−1420.

Matsumoto, Y., Satake, T., Shioji, N., & Sakuma, A. (1991). Inactivation of microorganisms by pulsed high voltage application. *IEEE Industry Applications Society Annual Meeting; Dearborn, MI* (pp. 652−659).

Roberts, W. A., Jr. (2003, Jul). Juiced Up. *Prepared Foods*, 23−24.

Roig, M. G., Rivera, Z. S., & Kennedy, J. F. (1995). A model study on rate of degradation of L-ascorbic acid during processing using home-produced juice concentrate. *International Journal of Food Sciences and Nutrition*, *46*, 107−115.

Schoenbach, K. H., Katsuki, S., Stark, R. H., Buescher, E. S., & Beebe, S. J. (2002). Bioelectrics — New applications for pulsed power technology. *IEEE Transactions on Plasma Science*, *30*(1), 293−300.

Sensoy, A., Zhang, Q. H., & Sastry, S. K. (1995). A high voltage electric field treatment system for preservation of electrically conductive foods. *IFT Annual Meeting Book of Abstracts, Paper 54A-18, 150*.

Uemura, K., & Isobe, S. (2003). Developing a new apparatus for inactivating *Bacillus subtilis* spore in orange juice with a high electric field AC under pressurized conditions. *Journal of Food Engineering*, *56*(4), 325−329.

Wouters, P. C., Dutreux, N., Smelt, J. P. P. M., & Lelieveld, H. L. M. (1999). Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. *Applied and Environmental Microbiology*, *65*(12), 5364−5371.

Zimmermann, U. (1986). Electrical breakdown, electropermeabilization and electrofusion. *Reviews of Physiology Biochemistry and Pharmacology*, *105*, 176−256.

Zimmermann, U., Pilwat, G., & Riemann, F. (1974). Dielectric breakdown of cell membranes. *Journal of Biophysical*, *14*, 881−899.